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The development of gut associated lymphoid tissue in the terminal ileum of fetal human intestine

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SUMMARY

Lymphoid tissue in formalin fixed and snap frozen human fetal ileum has been studied using immunohistochemistry. At 11 weeks gestation clusters of cells expressing CD4 (leu-3a positive) are present in fetal ileum but these do not express CD3 (UCHT1 negative) and are probably macrophages. Aggregates of lymphoid tissue are apparent from 14 weeks gestation which contain T cells of helper/inducer and suppressor/cytotoxic phenotype. Both B and T cells are present at 16 weeks but with no cellular zonation. By 19 weeks, distinct follicles of B cells are present surrounded by T cells of helper/inducer and suppressor/cytotoxic phenotype. Follicular dendritic cells are also present within the B cell areas. The B cells at this age express surface IgM and IgD, C3b- and C3d-receptors. They also express the antigen CD5 which has been shown by others to be present on some fetal B cells but which is almost exclusively associated with T cells in the adult. HLA-D region antigens are present on apparently all of the cells within the fetal lymphoid follicles. The antigen on activated B cells, CD23 (recognized by MHM6), was present on some cells scattered within the B cell follicle. This is indicative of antigen independent B cell proliferation.

Keywords gut-associated lymphoid tissue Peyer's patches fetal human intestine

INTRODUCTION

Peyer's patches form the bulk of the organized lymphoid tissue in the intestine of man and animals. Work in the sheep suggests that Peyer's patches may be sites of lymphopoesis *in utero*, and the large caecal Peyer's patch in pre- and postnatal lambs may be a primary lymphoid organ equivalent to the avian bursa of Fabricius (Reynolds & Morris, 1984). This is not likely to be the case in rodents because Peyer's patches undergo most of their development postnatally (Hummel, 1935; Mayrhofer, Pugh & Barclay, 1983). Little is known however of the development of lymphoid tissue in human fetal intestine.

Peyer's patches can be seen histologically in human fetal intestine from the beginning of the 5th month gestation (Baginsky, 1882; Cho, 1931) and lymphoid aggregates containing B and T cells have been described using immunohistochemistry in fetal intestine from 16 weeks gestation (Spencer *et al.*, submitted). Immunohistochemical and immunocytological studies of various human fetal lymphoid organs have shown that the B cells in primary and secondary lymphoid tissues are phenotypically distinct from each other, and in the case of the secondary lymphoid organs distinct from their equivalent in adult tissue. Pre-B cells in lymphopoeitic organs such as

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bone marrow characteristically express cytoplasmic IgM but not surface IgD, whereas fetal lymph nodes and spleen contain B cells which express both surface IgM and IgD and also the antigen recognized by UCHT2 (CD5) (Bofill *et al.*, 1985). The aim of this study was therefore to follow the structural development of Peyer's patches, to characterize the phenotype of the lymphoid cells forming aggregates in fetal human gut and relate these findings to the developing lymphoid system in the peripheral lymphoid organs of man and the gut associated lymphoid tissue of animals described by others.

MATERIALS AND METHODS

Collection of tissue. Ileum was obtained from spontaneously aborted fetuses which had been fixed in unbuffered formalin, or from therapeutic abortions by curretage in which case fragments of ileum were snap frozen in liquid nitrogen. The numbers of specimens of each age studied are shown in Table 1.

Table 1. Number of specimens of each age collected

	Number of specimens				
Type of specimen	11 weeks	12 weeks	14 weeks	16 weeks	19 weeks
Paraffin embedded tissue	—	—	14	7	6
Frozen tissue	1	1	2	4	1

Table 2. Details of monoclonal antibodies used in this study

Antibody	Source	Specificity	Reference
UCHT1	1.	T cells (CD3)	Beverley & Callard, 1981
UCHT2	1.	T cells and some B cells (CD5)	Martin *et al.*, 1981
UCHT4	1.	T cells (CD8)	
leu-3a	Becton Dickinson	T cells (CD4)	Engleman *et al.*, 1981
PD7/26	2.	leukocyte common antigen	Warnke *et al.*, 1983
Pan B	Dako	B cells	Stein *et al.*, 1982
IgM	Coulter	μ-chains	
IgD	Coulter	δ-chains	
E11	3.	C3b-receptors	Hogg *et al.*, 1984
B2	Coulter	C3d-receptors (CD21)	Nadler *et al.*, 1981
MHM6	4.	Activated B cells (CD23)	Rowe *et al.*, 1982
R4/23	2.	Follicular dendritic cells	Naiem *et al.*, 1983

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Immunohistochemistry. Paraffin sections of formalin fixed tissue were stained using immunoperoxidase as described elsewhere in detail (Isaacson & Wright, 1983). Optimal staining was achieved using trypsin digestion (Mepham, Frater & Mitchell, 1979).

Cryostat sections were cut at 8 μm, air dried for 30 min, fixed in fresh acetone for 30 min and stained using an indirect immunoperoxidase technique as described elsewhere in detail (Isaacson & Wright, 1983).

Monoclonal antibodies used, their source and specificity are shown in Table 2.

Quantitative studies. Sections of lymphoid follicles with optimal preservation of cellular morphology were selected for quantitative study. All of the cells in the follicles were counted, the numbers of cells present ranging from 25 to 260. The numbers of immunoperoxidase stained cells as percentages of the total number of cells in the follicles were calculated. The percentage of helper/inducer T cells was estimated by subtracting the percentage of cells expressing CD8 (suppressor/cytotoxic) from the total number of T cells expressing CD3. This avoided the complication caused by the presence of the antigen CD4 on cells of the macrophage/monocyte lineage as well as on the helper/inducer T cell subpopulation.

RESULTS

Formalin fixed terminal ileum from 27 fetuses ranging in age from 14 to 19 weeks was studied in paraffin sections stained with haematoxylin and eosin. Aggregates of lymphoid tissue identifiable by cellular morphology were not apparent until 16 weeks gestation. At 16 weeks, clusters of leucocytes with diverse morphological characteristics were seen adjacent to the epithelium (Fig. 1a). At 19 weeks larger aggregates of cells were present, again cells were morphologically diverse, with no obvious zonation (Fig. 1b). The lymphoid nature of the aggregates was confirmed using immunoperoxidase with the leucocyte common antibody PD7/26 (Fig. 1c). Cells with eosinophilic granules were present in the lamina propria of a third of the fetuses studied.

Paraffin embedded material is not suitable for analysis of surface markers and so to obtain more detailed information cryostat sections of ileum from nine fetuses ranging from 11 to 19 weeks gestation were also studied. The following observations were made:

(a) The 11 and 12 week old fetal guts were sectioned extensively but no aggregates of lymphocytes were seen. Some clusters of cells recognized by the antibody leu-3a were present but these were not stained in serial section with the pan T cell antibody and were probably macrophages (Wood, Warner & Warnke, 1983).

(b) The first aggregates of lymphoid tissue were observed, albeit infrequently, at 14 weeks. Most follicles contained T cells, but others contained both B and T cells with no structural separation. T cells of helper/inducer and suppressor/cytotoxic phenotype clearly expressed CD4 and CD8 respectively but the precise number of lymphocytes expressing CD4 was difficult to determine because this antigen is expressed on some other cells such as macrophages (Wood *et al.*, 1983). The number of helper/inducer T cells was estimated as the number of cells with CD3 present in excess of those with CD8 as explained in 'Materials and Methods'. T cells were found to be predominantly of helper phenotype (3:1, one follicle counted). B cells recognized by the pan B cell antibody were examined in serial section and were seen to express surface IgM and IgD. It was not possible to characterise these cells further because of their infrequent occurrence.

(c) At 16 weeks gestation larger aggregates of lymphoid tissue were observed consistently containing both B and T cells but with no cellular zonation (Fig. 2). The ratio of helper/inducer: suppressor/cytotoxic T cells determined as before varied from 0·5:1 to 6:1 (four follicles counted from three fetuses). The ratio of T:B cells also varied within and between individuals from 1:1 to 7:1 (eight follicles counted from four fetuses) in favour of the T cells. B cells expressed surface IgM and IgD, C3b- and C3d-receptors.

(d) At 19 weeks gestation the follicles were structured with distinct zonation of B and T cells (Fig. 2). Follicular dendritic cells recognized by the antibody R4/23 were also present and were restricted to the B cell zone of the follicles. T cells surrounding the follicle contained helper/inducer and suppressor/cytotoxic T cells in a ratio of 3:1 (2 follicles counted). The B cells expressed surface

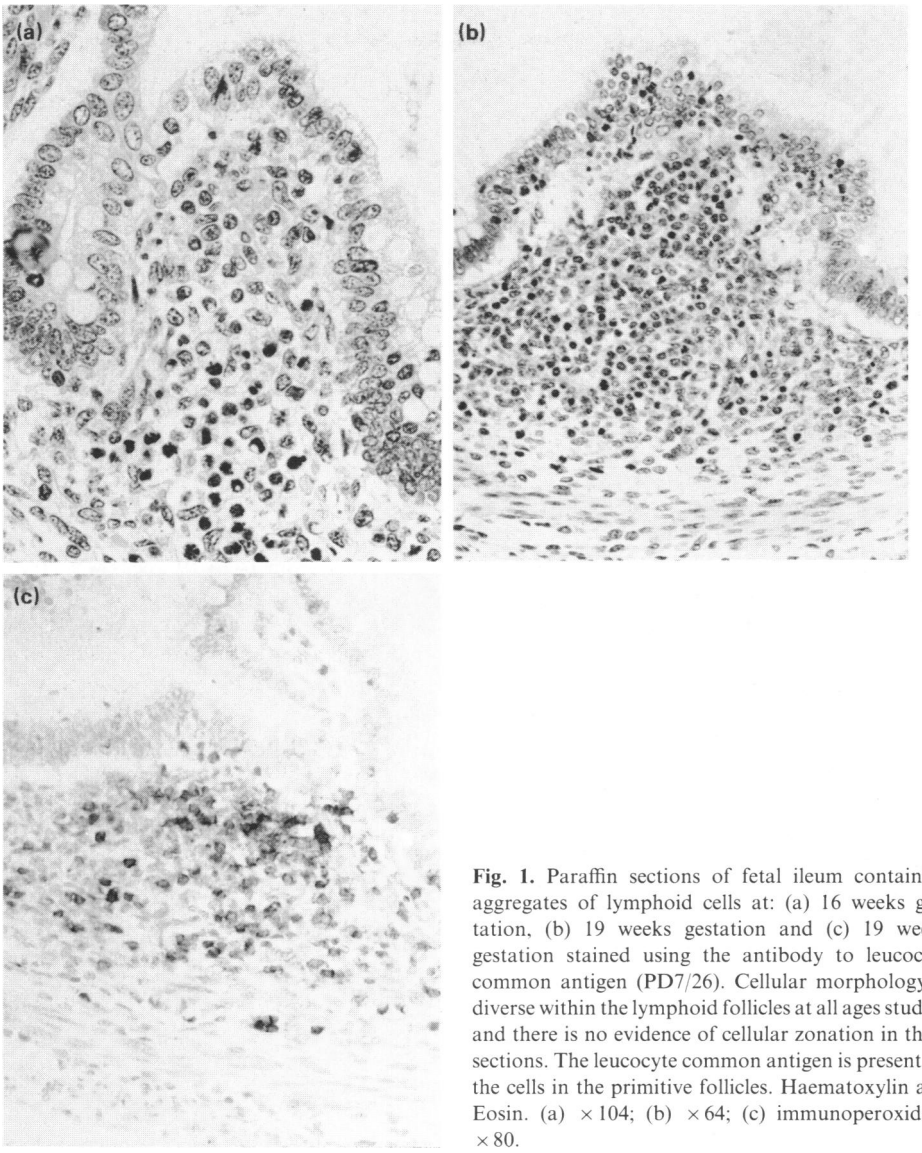

Fig. 1. Paraffin sections of fetal ileum containing aggregates of lymphoid cells at: (a) 16 weeks gestation, (b) 19 weeks gestation and (c) 19 weeks gestation stained using the antibody to leucocyte common antigen (PD7/26). Cellular morphology is diverse within the lymphoid follicles at all ages studied and there is no evidence of cellular zonation in these sections. The leucocyte common antigen is present on the cells in the primitive follicles. Haematoxylin and Eosin. (a) ×104; (b) ×64; (c) immunoperoxidase ×80.

IgM and IgD, C3b- and C3d-receptors, and also CD5. B cells stained less intensely with UCHT2 (CD5) than the adjacent T cells (Fig. 3). Cells stained by MHM6 which recognizes activated B cells were scattered within the B cell zone of the follicles.

(e) An interesting observation at 19 weeks was the appearance of cells in the lamina propria with endogenous peroxidase a characteristic of eosinophils. The presence of eosinophils in the 19 week old frozen fetal tissue was confirmed in sections stained with haematoxylin and eosin.

(f) All of the cells in follicles of all gestational ages were HLA-D region positive, regardless of cellular zonation. HLA-DP and DR were present from 11 weeks and DQ from 14 weeks.

DISCUSSION

This paper reports the first detailed analysis of the development of gut-associated lymphoid tissue in man. Signs of Peyer's patch development were first seen in a single specimen at 11 weeks gestation

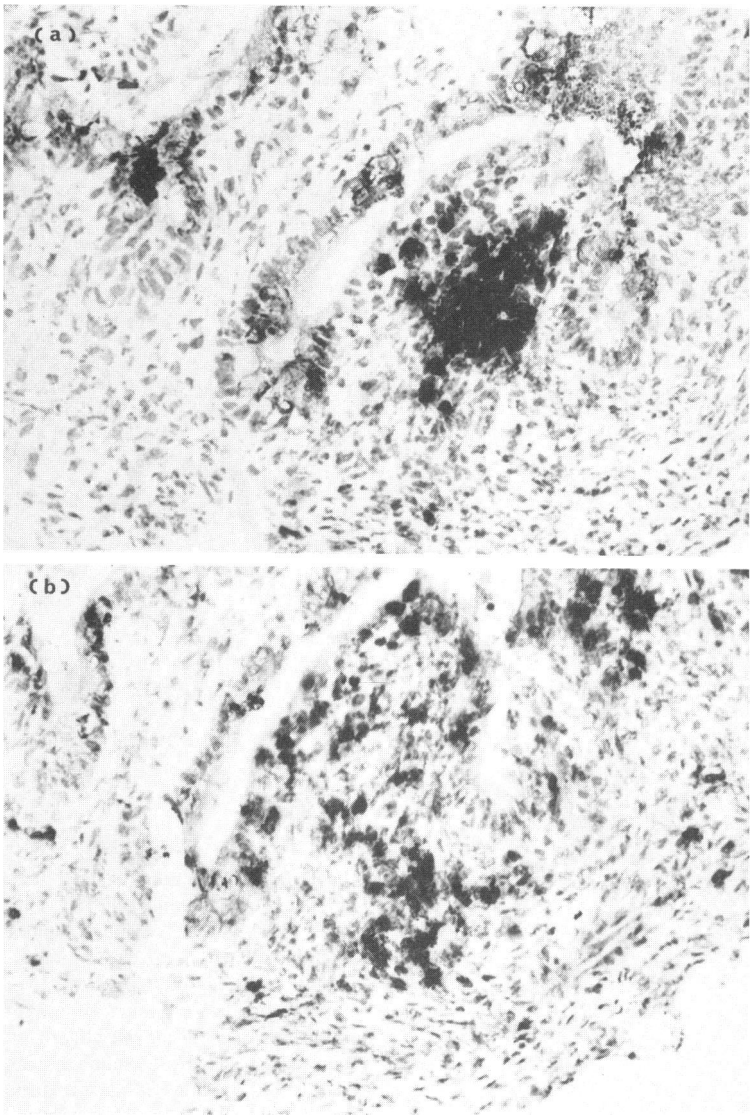

Fig. 2. Cryostat sections of fetal human ileum at 16 weeks gestation stained using primary antibodies to: (a) B cells and (b) T cells (UCHT1, CD3). The lymphoid aggregates contain both B and T cells at this age but with no distinct zonation. Immunoperoxidase $\times 80$.

when clusters of cells expressing CD4 were present adjacent to the epithelium. These cells were not T cells but probably macrophages which can also be CD4 positive (Wood *et al.*, 1983). The next cells to populate the follicles were T cells, and then B cells between 14 and 16 weeks gestation. The appearance of follicular dendritic cells at 19 weeks was concomitant with the appearance of tissue architecture consisting of a distinct T cell zone surrounding a B cell follicle. At this time the antigen CD5 recognized by UCHT2 was clearly apparent on the B cells. Thus in the 8 week span of fetal life studied, at 4 months before birth, the Peyer's patches develop from clusters of cells expressing CD4 into ordered structures of B and T cells very similar to those seen in adult tissue (Spencer *et al.*, 1986). These observations of structural development and B cell phenotype are consistent with those recently made by Bofill *et al.*, (1985) in fetal lymph node. This suggests that the cells in developing Peyer's patches are analogous to those in fetal lymph nodes and it is unlikely that they are unique

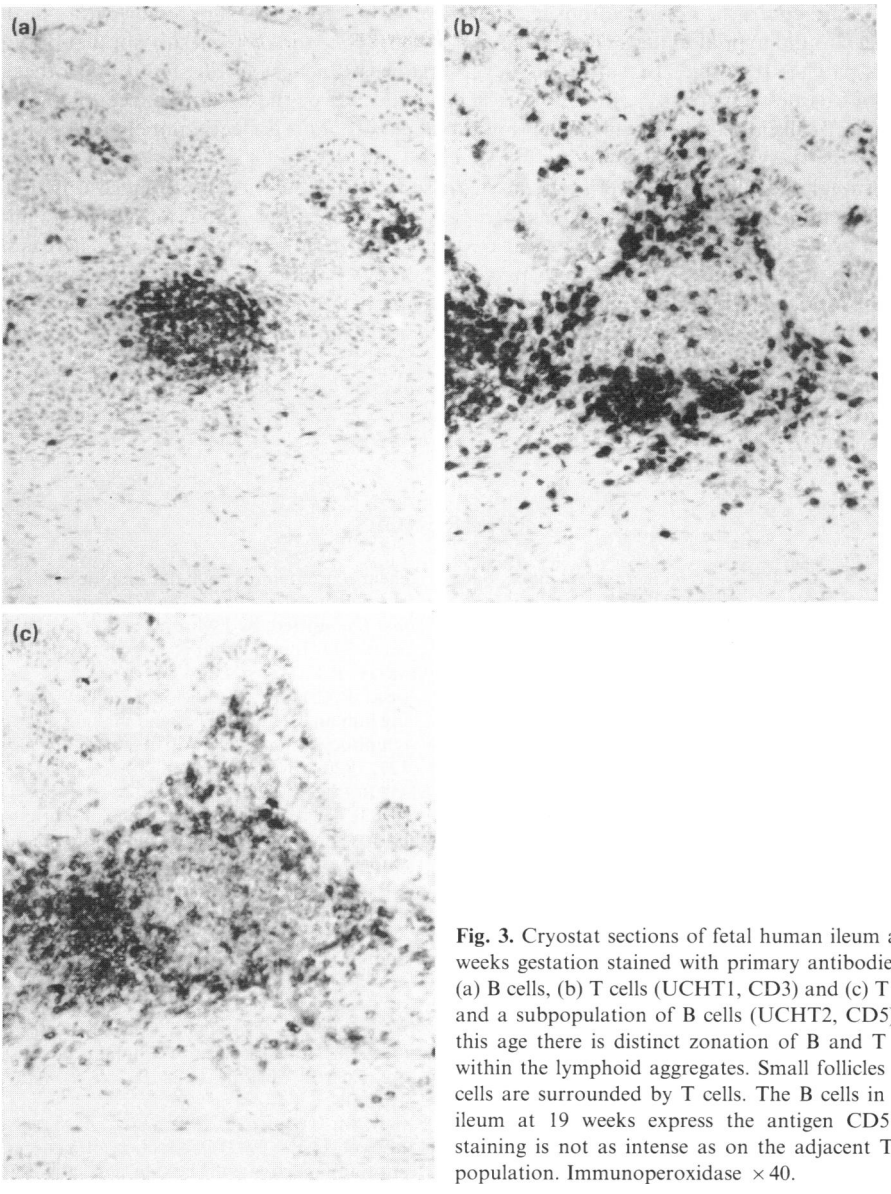

Fig. 3. Cryostat sections of fetal human ileum at 19 weeks gestation stained with primary antibodies to: (a) B cells, (b) T cells (UCHT1, CD3) and (c) T cells and a subpopulation of B cells (UCHT2, CD5). At this age there is distinct zonation of B and T cells within the lymphoid aggregates. Small follicles of B cells are surrounded by T cells. The B cells in fetal ileum at 19 weeks express the antigen CD5 but staining is not as intense as on the adjacent T cell population. Immunoperoxidase $\times 40$.

sites of lymphopoesis in the developing immune system. It is interesting to note however that occasional activated B cells recognized by MHM6 were present within the B cell zone. These cells are not seen in adult primary lymphoid follicles (only in follicle centres) and their presence in fetal B cell follicles suggests that there may be some B cell proliferation, which is either antigen independent or due to B cells recognizing self antigen.

The B cells with a phenotype of surface IgM^+ and IgD^+, C3b-receptor$^+$, $CD5^+$ seen in fetal B cell follicles is identical to that characteristic of malignant lymphoma known as B cell lymphoma-centrocytic (Stein *et al.*, 1984). This tumour has two forms, peripheral and intestinal. In the intestine it manifests as multiple lymphomatous polyposis (Cornes, 1961; Isaacson *et al.*, 1984). It is possible that the B cells forming follicles in fetal gut which have no equivalent in adult tissue represent the benign equivalent of the malignant cells in this lymphoma.

Our finding that the majority of T cells in fetal Peyer's patches is of helper/inducer phenotype

($CD8^+$) is in contrast to observations in fetal liver and spleen where suppressor/cytotoxic T cells have been found to predominate (Rosenthal *et al.*, 1983). The ratio of T cell sub-populations in fetal Peyer's patches resembles that in adult Peyer's patches (Spencer *et al.*, 1986) and other adult lymphoid tissues (Dvoretsky, *et al.*, 1982). The significance of the predominance of suppressor/cytotoxic T cells in fetal liver and spleen is not understood but it is clearly not characteristic of all fetal tissues.

Although the number of Peyer's patches increases with age (Cornes, 1965) suggesting that their development is at least partially dependent upon the presence of luminal antigens, a significant number are well established at birth having developed in a germ free environment. There is strong expression of HLA-DR by fetal Peyer's patch lymphocytes and eosinophils can be found in fetal lamina propria. These findings imply that there is a competant mucosal immune system present at birth to protect the newborn against pathogens in the developing enteric flora.

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